1. INTRODUCTION

1.1 Background

Biodeterioration has been defined as the undesirable changes in the properties or qualities of a material or a structure by the vital activities of organisms (Allsopp D. *et al*.,2004). The discoloration of the buildings and the damage to their structure is one of the most common manifestations of biodeterioration. In biodeterioration, bioreceptivity is one of the most important phenomena. The concept of bioreceptivity was defined by Guillitte (1995) as the aptitude of a material to be colonized by one or several groups of living organisms (Guillitte, 1995; Miller *et al*., 2012). Different materials can have different structures like being porous or semi porous and also varying surface features like being smooth or rough., Biological organisms such as cyanobacteria, micro green algae, bryophytes and lichens colonize and deteriorate the surface of the monuments or buildings which are made up of materials which are porous or semi-porous (Gil and Saiz-Jimenez, 1992, Adhikary and Kovacik, 2010; Ortega-Morales *et al*., 2013; Verma *et al*., 2014; Joshi *et al*., 2015; Nayak *et al*., 2017).

An ancient monument is any structure, erection or building or any cave, rock-sculpture which is of archaeological, historical or artistic interest and which has been in existence for not less than 100 years (Anon, 2010). India has a rich diversity of monuments and several ancient monuments of India are an inspiration for the present and future generations. It is important that such sites are properly preserved and maintained so that citizen can make full use of their social and cultural values. Among the many ancient monuments in India, several such important monuments are protected by the ‘Ancient Monuments and Archaeological Sites and Remains Act 1958 (Anon,2010). Monuments are generally endangered due to adverse environmental conditions like typically hot and humid surroundings that are common in a tropical country like India and this facilitates biodeterioration. Based on the environment and surface of the material, encouraging the microorganisms on it. Microorganisms like cyanobacteria, micro green algae and lichens are pioneering organisms (Adhikary and Kovacik, 2010 and Bajpai and Upreti, 2014) which colonize such monuments. The environmental conditions in the vicinity and the surface of the material that constitute the monument plays an important role in encouraging the growth of microorganisms on it. Over a period of time these sites are taken over by bryophytes and some allied vascular plants forming a seral community and ultimately leading to a climax community (Fig. 1.1). Climax community plants have root that penetrate and grow in between fissures which causes the structure to crack resulting in the deterioration of the structure (Crispim and Gaylarde, 2005). The Angkor Wat temple complex in Combodia is a classic example of community succession on monuments (Bartoli *et al.*, 2014).

Fig. 1.1 – Ecological succession on the monuments walls causing biodeterioration

The process of biodeterioration occurs in different ways in different rock or substratum types. The rock surface may be pitted, sandstone or granite may flake off in fairly large and flat pieces, the glaze on brick surface may crack while the cryptoendolithic growth of microorganisms in sandstone can result in the formation of blisters (Keshari and Adhikary, 2014). Preserving monuments becomes difficult and costly after such damage has been made to its fundamental structure. Hence, it is an important to stop the growth of the biological organisms in the initial phase resulting in better conservation of the monuments or structures. Therefore, it is needed to study the pioneering organisms, lichens and bryophytes diversity and their role towards deterioration. The current study focuses on four groups of organisms named cyanobacteria, micro green algae, bryophytes and lichens which are described individually later in this chapter. Their growth has been studies on selected monuments in Gujarat which have social, cultural and heritage importance.

1.1.1 Weathering and Biodeterioration

Biodeterioration occur due to a combination of physical weathering and biological weathering (Keshari and Adhikary, 2014). As fig 1.2 shows, several abiotic factors are responsible for physical weathering. Biological weathering manifests itself in two ways - mechanically and chemically. Structures like rhizines (lichens), rhizoids (bryophytes) or roots of biological organisms penetrate a few millimetres into the pores of the substratum. This penetration process take place through mechanical force (Gil and Saiz-Jimenez, 1992, Crispim and Gaylarde, 2005). In chemical biological weathering, the biological organisms secrete a variety of chemicals of which, some are secondary metabolites or acids that are harmful for the structure and causes deterioration (Crispim and Gaylarde, 2005; Bajpai and Upreti, 2014; Choudhury *et al*., 2016).

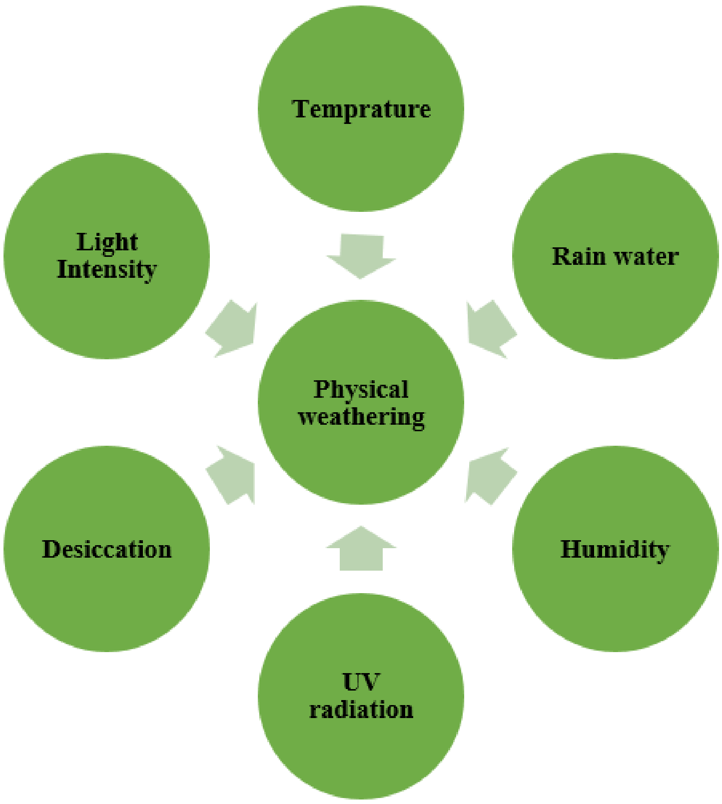

Fig. 1.2 – Factors responsible for Physical weathering in biodeterioration

1.2 Substratum material in biodeterioration of the monuments

India is rich in architectural heritage and this diversity of monuments have been built using different materials like sandstone, limestone, basalt, granite, marble, different materials in mortars and bricks (Sinha *et al*., 2004; Modi, 2008;). All these materials have different physical and chemical properties. The growth and survival of microorganisms depends to a large extent upon these properties of the substratum.

1.2.1 Physical properties of the substratum material

Physical properties depend primarily on the origin and history of the substratum material or rock used in the construction of the monuments. The sample of one type of rock obtained from different localities varies (Robertson, 1982). The most important physical properties of the substratum material (rock)from a biodeterioration perspective are porosity and permeability (Robertson, 1982; Gil and Saiz-Jimenez, 1992) because these properties regulate the accessibility of water to the interior of the stones. Porosity depends on the aggregation of the minerals and the sorting of the sand grains. The flow of water depends on the permeability of the substratum. Some other properties like hardness, strength, elasticity etc. are also play a role in substrate degradation as a part of biodeterioration.

1.2.2 Chemical properties of the substratum material

Chemical properties depend on the type of the stone. Different types of stones or rocks have different chemical properties based on their composition. For example, sedimentary rocks have granule-like structure in composition while metamorphic rocks have fine grained particles in composition. Igneous rocks on the other hand have different grain sizes from fine and equigranular to coarsely granular. Sandstone and limestone are types of sedimentary rocks, quartzite and marble belong to the metamorphic group while granite, rhyolite and basalt are from the igneous rock group. Sandstone has intergranular minerals like silica, limonite, calcite and clay while limestone usually contains quartz, clay and other silicate minerals. Basalt has minute grains of plagioclase feldspar and other minerals with less than 20% quartz. Rhyolite also has feldspar and quartz but with different proportion as compare to basalt (Herz, 1982). In Gujarat most of the monuments have been built using sandstone, limestone, basalt or rhyolite. Because all these types of rocks have different physical properties, any study on biodeterioration would be incomplete without information on the material composition of the monument.

1.3 Role of Cyanobacteria and lower plants in biodeterioration

Each group of biological organism have a different ability to grow and survive in harsh environmental conditions. Each group of organism also plays a different role in the deterioration of the substrate. The current study focuses on the micro green algae, bryophytes and lichens as lower plant group and the cyanobacteria. All these groups are individually explained in detail below.

1.3.1 Cyanobacteria and micro green algae

Cyanobacteria and micro green algae are phototrophic organisms that live in both aquatic and terrestrial habitats. Cyanobacteria and micro green algae have a colonial or filamentous form. Both of these groups have major differences which are given in below table. 1.1.

Table 1.1 – Important differences between cyanobacteria and micro green algae

Sr. No.	Character	Cyanobacteria	Micro green algae
1.	Type of organism	Prokaryotic	Eukaryotic
2.	Cell wall	Peptidoglycan	Cellulose
3.	DNA Structure	Naked DNA	In Nucleus and Chloroplast
4.	Cellular organelles	Absence of membrane bound organelles	Presence of membrane bound organelles
5.	Chloroplast	Absent	One or more chloroplast per cells
6.	Pigments	Chlorophyll a, phycoerythrin and phycocyanin	Chlorophyll a, Chlorophyll b, carotenoids and xanthophyll
7.	Nitrogen fixation	Involved	Not involved
8.	Asexual reproduction	Fission, budding, fragmentation or formation of zoospore	Cell division and fragmentation with or without formation of small segments called hormogones (hormogonia), hormospores, akinetes, endospores, nanocytes or exospores
9.	Sexually reproduction	Absent	Present

(Compiled from various sources)

1.3.1.1 Cyanobacteria

The cyanobacteria have been established as a phylogenetically coherent group of evolutionary ancient, morphologically diverse and ecologically important group (Garcia-Pichel, 2009). Cyanobacteria members have a sheath on the outer side of the cells in both the colonial and filamentous forms. Each filament consists of one or more cellular strands called trichomes. A Single filament could be either homocystous and heterocystous. A homocystous filament has the same type of cells while the hetercystous filament has differentiated cells which are known as heterocysts (Sharma *et al*., 2014). The main morphological characteristics that help in diagnostic of the families were given in a concise form by Anagnostidis and Komarek (1990) and Komarek and Anagnostidis (1989) and have been updated by Komarek and Anagnostidis (1999) and Komarek *et al*., (2013). Cyanobacteria are an especially challenging group to classify because of their long and arguably complex evolutionary history. Higher taxa were defined by some morphological characters like coccal vs trichal form, tapering, polarity, types of branching, dimensions, presence of akinetes etc. But all these characters have apparently arisen and /or been lost several times during the evolution of modern species and genera (Gugger and Hoffmann, 2004; Schirrmeister *et al*., 2011; Komarek *et al*., 2013; Shih *et al*., 2013). Hence, a polyphasic approach is considered necessary for cyanobacterial identification (Komarek, 2016). The 16S rRNA gene sequences are broadly used for molecular identification.

1.3.1.2 Micro green algae

Micro green algae have a large diversity of species which are classified under many different orders. But from a biodeterioration perspective, members of Chlorococcales are mostly involved in the phenomena (Adhikary and Kovacik, 2010). Members of this order are either unicellular or multicellular. Some members of this order also show the presence of polysaccharides sheaths on the outer side of the cells. Due to this, coccoid cells are lumped together and form many groups (Komarek and Fott, 1983).

1.3.1.3 Biofilm formation and Extracellular polysaccharides substances (EPS)

Members of cyanobacteria and micro green algae can survive in a large variety of environmental conditions like low or high temperatures, high light intensities, pH and salinity. Due to these qualities, several phototrophic cyanobacteria and micro green algae grow and form greenish or blackish biofilm on the exposed surface of the substratum

(Crispim and Gaylarde, 2005). Cyanobacteria are a key player in forming biofilm and deteriorating the substratum. The biofilm is comprised of one or more types of microorganisms collectively growing on the surface. The biofilm microorganisms secret polymeric substances on the outer side of the cells. Such substance is known as extracellular polymeric substances (EPS). They have a complex structures having exopolysaccharides, proteins and exo DNA (Nwodo *et al*., 2012). Among these, the exopolysaccharides are the chief biofilm forming component. Extracellular polysaccharides are very slimy in nature and are mainly composed of heteropolysachhrides or homopolysachchrides. Other microorganisms are embedded in it and help form complex biofilms (Wingender *et al*., 1999). The biofilm protects the cells against the harsh environmental conditions and plays an important role in biodeterioration.

1.3.2 Bryophytes

Bryophytes are the second largest group of land plants after flowering plants. They are essentially small plants ranging from a few millimetres to a few centimetres. The bryophytes are the only group of land plants that have a dominant gametophyte plant body that is independent and autotrophic. The gametophyte plant is either a dorsiventral thalloid or a leafy structure differentiated into stem-like and leaves-like structures, however, roots are absent. Instead of root, rhizoids are presents. The sporophyte stage is dependent on the gametophyte for nutrition and physical support. It is differentiated into foot, seta and capsule (Sharma, 2014). The spore producing structure is the capsule. It is generally a simple spherical structure embedded in gametophyte and capsule projects out of gametophyte by means of stalk like structure, the seta. The sporophyte derives its nutrition from the gametophyte with the help of specialized haustorial cells of foot. Bryophytes have capacity for regeneration. All spores of bryophytes are homosporous (Rashid, 2007).

Bryophytes are broadly divided into three major groups viz., liverworts, hornworts and mosses. From a biodeterioration perspective, the liverworts and mosses are found on the walls and other parts of the building or monuments. Hornworts generally grow on soil and do not play a role in biodeterioration of the wall structure. They are however important in monument locations where soil accumulates. Mosses are differentiated from other two groups of bryophytes by the presence of a distinct juvenile phase, ‘the protonema stage’ in their gametophyte phase. The protonema stage is the initial phase that covers the walls. Mature gametophyte has the leaf, stem and rhizoids. Rhizoids penetrate into the pores and fissures of the substratum (Gil and Saiz-Jimenez, 1992). Due to this, mechanical forces are generated which result in formation of cracks in their surroundings resulting in damage and deterioration of the wall structure.

The bryophytes have been historically classified into the orders Hepatica, Anthocerotae and Musci for the three major groups. Rothmaler (1951) suggested the names Hepaticopsida for Hepaticae, Anthocerotopsida for Anthocerotae and Bryopsida for Musci. Takhtajan (1953), Schuster (1958) and Udar (1975) classified Bryophyta into three divisions viz Hepaticopsida, Anthocerotopsida and Bryopsida. In recent, all the known bryophytes are grouped under three phyla: Marchantiophyta, Bryophyta and Anthocerotophyta. According to Stotler *et al*., (2008) Marchantiophyta has been classified into three classes. Bryophyta has been classified into five classes like Takakiopsida, Sphagnopsida, Andreaeopsida, Andreaeobryopsida and Polytrychopsida by Buck and Goffinet (2000). The classification of bryophytes is undergoing constant revisions and this revision is based on several adaptations such as special reproductive structures, alternation of generation, desiccation tolerance capacity etc. that help to survive in any harsh environment condition by Renzaglia *et al*., 2000.

1.3.2.2 Mineral uptake as Calcium uptake

The source of nutrients for bryophytes are precipitation, dust and substrate. (Glime, 2013). From the substrate the bryophytes uptake some nutrient for their growth due to which it causes degradation of the substrate. The cell walls of the bryophytes can exchange cation with their surroundings. This enables bryophytes to uptake essential cations like Ca^+ into the extracellular or intracellular region of the protoplast. Calcium held on the exchange sites is thought to influence the permeability of the adjacent plasmalemma. The level of exchangeable Ca^+ have been reported to be 16-17 times higher in calcicole mosses compared to calcifuges mosses (Bates, 1982).

1.3.3. Lichens

Lichens are made up of two different group of organisms viz. algae and fungi. Algae (autotroph) make the food for fungi while fungi give them substratum to live and protect against light and drought. In this mutual symbiotic relationship, the fungal partner is known as the mycobiont while the autotroph is known as the photobiont (Ayub, 2005). Green algae and cyanobacteria are the most common photobionts but in some lichen species, the mycobionts associate with brown algae (Phaeophyceae) or golden algae (Chrysophyceae). Lichens having green algae as the photobiont are known as chlorolichens while lichens having cyanobacteria as the photobiont are known as cyanolichens. Sometimes both photobiont green algae and cyanobacteria are incorporated with the same mycobiont. More than 98% mycobiont forming the lichens are members of the Ascomycetes. The remaining lichen forming fungal species are classified within the Basidiomycota (Bajpai and Upreti, 2014). Lichen classification and name are given based on the fungal classification.

Lichens are of five types based on their growth form viz. crustose, leprose, squmulose, foliose and fruticose. Crustose and leprose lichens are tightly attached to the substrate in comparison to the other lichen forms. Due to this, the mycobiont hyphae and rhizines penetrate into the rock surface and causes deterioration of the substrate. Lichens are also known to play an important role in pedogenesis (Chen *et al*., 2000). Respiratory CO_2 from lichens dissolved with water in the lichen tissues resulting in the formation of carbonic acids which enhance the solubility of rock surfaces by lowering the pH of the substratum (Seaward, 2001). Lichens also have secondary metabolites and acids that deteriorate the substrate.

1.3.3.2 Lichen secondary metabolites and acid harmful to the substrate

Lichens acids have a relatively low solubility but they are effective chelators forming metal complexes with silicates and other minerals derived from the substratum. They absorb moisture in liquid or vapour form and are known to retain in it even under extremely dry conditions. The medulla of the thallus acts as a water reservoir (Gehrmann *et al*., 1988). This water retaining capacity of lichens, offer more time for carrying out chemical reaction because the rock – lichen interference remains moist for longer periods. The metabolites of lichens react with stone materials and decompose them either through salts or chelate formation. Monovalent ions like Na^+ and K^+ of stone minerals get replaced by protons that are leached out by water movement. Bivalent or trivalent cations like Ca^{++}, Mg^{++}, Fe^{++}, Fe^{+++} are dissolved by chelate formation. The biogenically produced aliphatic carboxylic acids and aromatic phenolic acids chelate K, Na, Mg, Ca and Si of rocks and accelerate the deterioration of the substrate (Bajpai and Upreti, 2014). Moreover, oxalic acids secreted by the mycobiont acts as chelator of metal ions and reacts with the rock forming magnesium oxalate and calcium oxalate. Calcium oxalate exists in two hydrated forms, monoclinic monohydrate (whewellite) and tetragonal dihydrate (weddellite). The monohydrate form is the major biodeterioration product and the ratio between this and the dihydrate form is dependent on various environmental factors (Seaward, 2001).

1.4 Control measures

Control of biodeteriogens on the monuments are very important. Because of these biodeteriogens monuments are damaged and ruin its aesthetic, architectural and economic value. There are three types of control measures such as physical, mechanical and chemical which are briefly described below.

1.4.1 Physical treatment

In this treatment, different intensities and types of UV light for different hours were used (Bajpai and Upreti, 2014; Borderie *et al*., 2015).

1.4.2 Mechanical treatment

In this method, the pressure washer was used for cleaning facades and buildings. In another method, plants were removed completely from the monuments mechanically (Agrawal *et al*., 1994).

1.4.3 Chemical treatment

In this treatment biocides or herbicides were used for controlling the biodeteriogens on the monuments (Dewey, 1999; Pinna *et al*., 2012; Kakakhel *et al*., 2019).

1.5 Rationale of the study

The state of Gujarat is rich in its cultural heritage and monuments. Among these monuments, some important ones are protected by the ASI. All these monuments are in different phases of conservation. While some monuments are degraded, some others have been restored and are in a good state of preservation. Anthropogenic activity like damage by tourists or activists as well as the activity of biological organisms invading on the structure are also causing the degradation of the monuments over period of time. At some places restoration was done by physical method of plugging out the plants. Very few monuments have been restored employing scientific approach using chemical treatment for conservation and preservation of the monuments. Such an approach needs a sound scientific background and information on the diversity of organisms and the mechanisms by which they causing the biodeterioration of the monuments. This would immensely help conservation agencies like the ASI which can put in more effective, long lasting and sustainable conservation measures. Hence, the present study was carried out to achieve this goal.

1.6 Objectives for present study

The ancient monuments are losing their aesthetic and archaeological value due to biodeterioration. To understand biodeterioration, it is important to study the diversity of biofoulants and the role they play during the process of deterioration. With an aim to ensure the effective conservation of important monuments, this study has been proposed with the following objectives,

1. Identification of the diversity of biofouling lower plants and cyanobacteria at the selected sites using morpho-taxonomic as well as modern methods.
2. Analysing the specificity of biofoulants for specific geological substrates.
3. To study the specific role of different biofoulants on the process of biodeterioration.
4. To suggest measures for the control of these biodeteriogens.